REVIEW

Pharmacokinetic and Pharmacodynamic Considerations in the Design of Therapeutic Antibodies

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The design and development of therapeutic monoclonal antibodies (mAbs) through optimizing their pharmacokinetic (PK) and pharmacodynamic (PD) properties is crucial to improve efficacy while minimizing adverse events. Many of these properties are interdependent, which highlights the inherent challenges in therapeutic antibody design, where improving one antibody property can sometimes lead to changes in others. Here, we discuss optimization approaches for PK/PD properties of therapeutic mAbs.

The design and development of therapeutic monoclonal antibodies (mAbs), be it for improving the activity of existing therapeutic mAbs or bringing new therapeutics to patients, have advantages over other non-mAb therapeutic modalities. The improvements can be accomplished by optimizing the pharmacokinetic (PK) and pharmacodynamic (PD) properties of therapeutic antibodies, which can improve efficacy while minimizing adverse events and enhancing patient experience and compliance.[1] Because immunoglobulin G (IgG) is the most commonly used structural format in antibody therapeutics, this review will focus on tailoring both the PK and the PD properties of IgG antibodies to optimize antibody design.

Detailed knowledge of antibody structure and function allows PK/PD scientists to guide engineering of antibodies to address PK/PD-related issues. The PK/PD behavior of therapeutic antibodies is impacted by numerous factors, including antigen-binding specificity and affinity, molecular size, format, physicochemical properties, folding stability, solubility, effector functions, and the pharmacological activities mediated by the antibody. For example, having an in-depth understanding of the interaction of the variable and constant (Fc) regions of antibodies with their binding partners has allowed the engineering of antibodies with optimal pharmacological properties, such as enhanced target specificity and effector functions.[2,3] Furthermore, understanding how antibody charge and glycosylation impact antibody PK is key to designing antibodies that are more homogeneous and stable and have optimal clearance and desired effector functions for their therapeutic application. The fact that many of those properties are interdependent highlights the inherent challenges in therapeutic antibody design, in which improving one antibody property can sometimes lead to defects in others. In addition to improving inherent qualities, the introduction of new activities through novel scaffolds serves to similarly enhance antibody potency and function by altering aspects of their PK/PD properties. The scope of

this review is limited to therapeutic mAbs that are primarily based on an unconjugated whole IgG backbone.

IMPROVING PK THROUGH ANTIBODY DESIGN
The neonatal Fc receptor

Modifying the clearance of antibodies to achieve increased drug exposure and serum half-life offers numerous advantages, including reduced dose or less frequent dosing and potentially lower cost.[3] The serum half-life of human IgG antibodies, which averages 21 days for subclasses 1, 2, and 4, is long and one of the advantages of using IgGs as a therapeutic. Although this review is focused on human IgGs, it should be clarified that murine IgGs, which generally are the initial IgGs used in the early phases of biotherapeutic development (prior to humanization), have different nomenclature and function compared with human IgGs (**Table 1**). For IgGs (human or murine), the long half-life is predominantly governed by the interaction with the recycling neonatal Fc receptor (FcRn).[4] FcRn binds to the CH2-CH3 domain of the IgG with a stoichiometry of two FcRn molecules per IgG antibody. FcRn is a membrane-associated receptor that is structurally related to major histocompatibility complex class I molecules. FcRn is expressed in the mammary gland, placenta, kidneys, brain, eyes, liver, and skin, as well as by intestinal epithelial cells, endothelial cells, macrophages, monocytes, and dendritic cells. Key functions of FcRn from a PK/PD perspective include recycling of the therapeutic mAbs (long half-life), IgG transport (distribution in tissues), and antigen presentation of multimeric immune-complexes (may impact immunogenicity).[5,6] Following nonspecific pinocytosis, IgG is internalized into endosomes where it binds FcRn and is protected from lysosomal degradation. Subsequently, the FcRn-bound IgG is recycled and released extracellularly into systemic circulation resulting in long serum half-life, whereas IgGs that do not bind to FcRn are degraded by endosomal proteases.[6] The interaction between IgG and FcRn is strictly

pH-dependent, which is critical for IgG recycling and the long half-life. IgG binds to FcRn at the endosomal acidic pH (6.0–6.5) with nanomolar (nM) affinity, whereas negligible binding occurs at a physiological pH of 7.4. This lack of binding at pH 7.4 results in IgG-FcRn complex dissociation at the cell surface neutral pH (7.0–7.4) and release of IgG for continuing circulation.[7]

It is important to note that human IgG binds murine FcRn with higher affinity at acidic pH compared with murine IgG in wild-type mice, which should be taken into consideration when PK properties of human therapeutic IgGs are evaluated in rodents.[8] Cynomolgus monkeys have been shown to be more reliable surrogates for predicting human clearance of therapeutic human or humanized mAbs that are mediated by FcRn-mediated proteolysis.[9] The use of humanized FcRn transgenic mice has also been recently evaluated for this purpose.[10,11] Modulating the FcRn–IgG interaction through

Table 1 Human IgG subclasses and corresponding "functional" murine subclasses[a73,114]

Human	Murine
IgG1	IgG2a
IgG2	IgG3
IgG3	IgG2b
IgG4	IgG1

IgG, immunoglobulin.
[a]The human subclasses were numbered based on their abundance. The basis for the murine nomenclature is not clear.

Table 2 Examples of mutations in the CH2/CH3 interdomain region of mAb Fc that increase or decrease FcRn binding affinity[58,115]

Goal	AA	Mutations
Increase FcRn binding	Ala330	Ala330Val
	Ala378	Ala378Val/Thr
	Asp312	Asp312Ala
	Asn315, 361	Asn315Asp and Asn361Asp
	Asn434	Asn434Ala
	Gln311	Gln311Ala
	Glu380,382	Glu380Ala and Glu382Ala
	Met252	Met252Tyr[a]
	Phe241	Phe241Leu
	Pro230	Pro230Ser/Thr
	Pro238	Pro238Ala
	Ser254	Ser254Thr[a]
	Thr256,307	Thr256Ala and Thr307Ala
	Val264	Val264Ala/Glu
	Thr256	Thr256Glu[a]
Decrease FcRn binding	His310, 435	His310Ala and His435Ala
	Ile253	Ile253Ala
	Ser254	Ser254Ala
	Tyr436	Tyr436Ala

AA, amino acid; FcRn, recycling neonatal Fc receptor; mAb, monoclonal antibody.
[a]Met(M)252Tyr(Y)/Ser(S)254Thr(T)/Thr(T)256Glu(E) is the triple mutation known as YTE.

point mutations allows altering the PK profile of therapeutic antibodies by enhancing/reducing their half-lives. **Table 2** includes examples of mutations in the CH2/CH3 region of mAb Fc that increase or decrease FcRn binding affinity. The YTE mutant is the only mutant that has been tested clinically so far. Motavizumab-YTE showed an impressive decrease in clearance (71–86%) and a twofold to fourfold increase in half-life compared with Motavizumab in healthy volunteers.[12]

IgG-FcRn interactions are believed to be restricted to the Fc region; however, a recent report suggested that Fab regions influence FcRn binding due to the conformational flexibility of the Fab arms.[7] This observation was also supported by Schlothauer et al.[13] who showed that IgGs with identical Fc regions differed in their dissociation from FcRn, which suggested that the Fab region influenced FcRn binding. It was also shown that the charge distribution in the distal variable fragment (Fv) of IgGs is involved in binding to FcRn, leading to reduced terminal half-lives.[7] It is still not entirely clear whether the faster clearance of these antibodies is due to FcRn-mediated effects or due to nonspecific binding to tissues.[14]

Another important consideration when modifying the mAb binding to FcRn is that engineering IgGs with excessive binding to FcRn at both pH 6.0 and pH 7.4 (i.e., reduced pH dependence) can exhibit fast clearance.[15] Using a panel of novel Fc variants with high affinity binding at acidic pH and various affinities at pH 7.4, Borrok et al.[16] demonstrated an affinity threshold of neutral pH binding that limits efficient IgG recycling. Increasing neutral FcRn affinity binding beyond this threshold reduces half-lives and offsets the benefits of increased acidic binding. This highlights the inherent limits of FcRn-mediated half-life extension and its dependence on pH binding.

Other work in the modification of FcRn binding has focused on understanding the impact of FcRn binding on s.c. bioavailability. FcRn is expressed not only by immune cells but also by blood vessel endothelial cells in the skin, and can thus influence s.c. bioavailability by contributing to the rate and extent of presystemic IgG catabolism.[17] Deng et al.[18] showed that the s.c. bioavailability of murine IgG2a was affected by mouse FcRn-binding affinity. Upon examining three antiamyloid β mAbs with different binding affinities to mouse FcRn, the authors found that loss of FcRn binding at either pH 6.0 or pH 7.4 led to loss of the FcRn-mediated protection from catabolism at the absorption site and subsequently the lowest bioavailability (41.8%) as compared with the wild-type mAb. On the other hand, using mAbs engineered with the Thr250Gln/Met428Leu Fc mutations that improve their FcRn interactions seems to improve clearance but not s.c. bioavailability in cynomolgus monkeys.[19] These results suggest that other factors may have a stronger influence and also highlight that additional studies are needed to understand the role of FcRn, if any, and its impact on s.c. bioavailability.

The role of FcRn in mediating IgG clearance is not fully understood in other types of tissues, such as the brain or the eyes. FcRn seems to play a role in the clearance of amyloid beta-peptide (Aβ)–specific IgG complexes across the blood brain barrier (BBB).[20] However, FcRn does not seem to contribute significantly to the BBB transcytosis,

because intravenously-administered IgG1 displayed similar brain-to-plasma concentration ratios in FcRn-deficient mice and wild-type mice.[21] The impact of FcRn on IgG disposition in the ocular space is equally confounding to that of the brain. FcRn has been shown to be expressed by the ciliary epithelium and retinal and choroidal vasculature, which may affect the clearance of IgGs administered into the vitreous of the eye.[22] One role for FcRn has been demonstrated in transporting full-length IgGs administered into the vitreous across the blood-retina barrier in rats.[23] Furthermore, others have shown that IgG and IgG-FcRn null (mAb with Fc mutations that prevent binding to FcRn) show similar kinetics in the rabbit eye after intravitreal injection.[24]

pH-dependent antigen binding

Fukuzawa and colleagues[25] have developed recycling antibody or antibody with pH-dependent antigen binding through variable region engineering to improve the PK/PD profile of IgG by increasing the clearance of the antigen but not the antibody. When coupled with sweeping technology (through constant region engineering), which has increased FcRn binding at neutral pH, an enhanced FcRn-mediated uptake of the antibody–antigen complex into the endosome can be achieved and, thereby, an increase in the number of cycles of antigen binding and lysosomal degradation.[26] Igawa et al.[27] generated a sweeping antibody variant of tocilizumab, an antibody against the soluble and membranous interleukin-6 receptor (IL-6R). This increased the number of cycles of antigen binding and lysosomal degradation, which, coupled with Fc engineering to increase binding to FcRn, significantly improved both PK and PD in cynomolgus monkeys. Additionally, they showed that the Fc gamma receptor FcγRIIb is a major contributor in the cellular uptake of monomeric immune complexes.[27] Subsequently, the authors expanded the sweeping technology to accelerate the clearance of pH-dependent anti-IL-6R by engineering the Fc to selectively enhance human FcγRIIb (instead of FcRn) binding. In absence of concomitant Fc engineering, similar results were still achieved with recycling technology in a hypercholesterolemia antibody (anti-PCSK9).[28]

Isoelectric point and charge.

Charged amino acids within the complex IgG structure are not only crucial determinants of protein–protein interactions of IgG necessary for biological activity but also determinants of PK.[29,30] On the same lines, the overall charge of the mAb as measured by the isoelectric point (pI) has also been shown to influence both serum clearance and tissue. Furthermore, recent studies have suggested that the electrostatic interaction of antibodies is influenced by both the overall net charge and the distribution of the charges on the surface.[31–33]

Anionization (reducing pI) partially abrogates the interaction with cell surfaces and extracellular matrices, reducing nonspecific tissue uptake and increasing serum half-life. In the study by Igawa et al [30] the authors demonstrated that lowering the pI by engineering the variable region could reduce the elimination of IgG antibodies. In another report, it seemed that antibodies with lower pI values exhibited slower systemic clearance and higher s.c. bioavailability in both humans and minipigs compared with antibodies with higher pI values (> 9).[32] However, this study was limited by the number of antibodies studied. On the other hand, Khawli et al.[34] isolated the acidic (pI: 8.7–8.9), basic (pI: 9.16), and main (pI: 9) components of a humanized IgG1 mAb and observed no differences in serum PK in rats, suggesting that the pI differences among the fractions were not sufficient to induce measurable changes in PK.

Conversely, IgGs with increased pI (cationization) display increased plasma clearance, volume of distribution, and tissue uptake, most likely due to enhanced binding to negatively charged cell surface molecules (heparin sulfate proteoglycans and phospholipids) and pinocytic uptake.[35] Cationization by converting surface carboxyl groups to positively charged groups, such as primary amines, has been used to improve uptake of IgG in target tissues (such as crossing the BBB).[36,37] However, the advantages of cationization must be interpreted with caution due to additional consequences of IgG retention in nontarget tissues. For example, a study of antihepatitis C virus E2-glycoprotein antibody variants showed very fast clearance for a variant with a pI of 8.61 compared with a variant with a pI of 6.10. Although both variants had comparable FcRn binding, the high pI variant was catabolized in the liver and spleen, suggesting that antibody charge can influence antibody catabolism independently of FcRn-mediated recycling.[29]

As noted previously, reducing or increasing the pI can have notable impact on mAb PKs. However, improved PK properties have also been demonstrated through charge modifications (with no demonstrable impact on overall pI) being applied to charge patches (clustering of charged amino acid residues) through either substitutions of charged amino acids or direct chemical modification of amino acid side chains with positive, negative, or neutral chemical groups.[35] Datta-Mannan et al.[38] studied two humanized mAbs with unexpectedly rapid clearance in mice and attributed their fast clearance to solvent-exposed positive-charge patches on the complementarity-determining region (CDR). Rationally balancing the CDR charge without affecting pI yielded significant improvement in peripheral exposure and reduced tissue catabolism. Bumbaca Yadav et al.[31] studied the impact of charge on the PK as well as s.c. bioavailability of two mAbs targeting lymphotoxin alpha (anti-LT-α) and human epidermal growth factor receptor 2 (anti-HER2). The authors engineered the Fv region to have either high or low Fv charge as compared with the parent antibody, with only a minimal change (< 1 pI unit) in pI measured for the whole antibodies. PK studies in cynomolgus monkeys demonstrated that high Fv charge mutants displayed faster clearance, whereas low Fv charge mutants displayed either no change (anti-HER2) or decreased (anti-LT-α) clearance. The impact on s.c. bioavailability was also striking, with increased bioavailability observed for the low Fv charge mutant of anti-HER2 and decreased bioavailability observed for the high Fv charge mutant of anti-LT-α. Similar PK profiles were observed in rats and mice.[31]

Because the charge of variable region has impact on the PK properties, attempts have been made to use surface charge to predict antibody clearance. Sharma et al.[39] used the net charge of the Fv domain at pH 5.5 as a criteria to identify antibodies with fast clearance in cynomolgus monkeys (i.e., > 10 mL/day/kg). While achieving ~70% accuracy of prediction, this approach did not incorporate other critical factors that affect antibody PK/PD (e.g., structure, charge distribution). Alternative approaches, such as developing a quantitative structure-activity relationship model using mutational charge distributions from a single IgG framework, might offer better predictions.

Affinity. High-affinity antibodies (K_d < 1 nM) are desirable not only for their assumed enhanced potency and efficacy but also for reducing the dose and the frequency of administration.[40–42] The delicate balance that exists among conformational stability, solubility, and high affinity highlights the challenge of rationally designing antibodies with engineering strategies that allow the prediction and control of this balance. The desired affinity for an antibody depends on several factors, including antigen density and turnover, mechanism of action (for example, agonistic or antagonistic antibody), dose, drug concentrations that can be achieved at the site of action, and the desired pharmacological effect. For example, Putnam et al.[43] studied high-affinity anti-IgE antibody-1 (HAE1), a high-affinity version of the US Food and Drug Administration–approved anti-IgE mAb omalizumab with the same IgG1 framework but different CDRs. A change of nine CDR residues reduced k_{off} of HAE1 to IgE by ~22-fold and enhanced the binding affinity to IgE by ~23-fold. In a subsequent PK/PD study in cynomolgus monkeys, HAE1 had a similar PK profile to that previously generated with omalizumab but required lower concentrations to suppress free serum IgG levels, suggesting that the higher affinity of HAE1 may translate into greater *in vivo* potency.[44] PK/PD modeling can be used to determine the optimal affinity range provided when information about the antigen kinetics, dose range, and human PK is available.[45,46] The avidity of the antibody to its receptor can also be leveraged to improve potency.[47,48]

Enhancing the affinity of an antibody for its target must be balanced with potential increases in nonspecific binding to various tissues, which may counterbalance expected improvements in PK/PD.[49] Wu et al.[49] applied a directed evolution approach to improve both the association rate (k_{on}) and dissociation rate (k_{off}) of palivizumab to the RSV F protein and found that raising the affinity by increasing k_{on} improved virus neutralization for the IgG form of the mAb. However, a subsequent evaluation of several of these affinity-optimized variants found that k_{on}-driven mutations generated nonspecific binding to various tissues that led to poor serum PK and lung bioavailability. Reverting three of the affinity-matured amino acids to the original sequences greatly reduced nonspecific binding and resulted in motavizumab, which binds to the RSV F protein 70-fold better than palivizumab.

Although this review is primarily focused on IgG1 therapeutic antibodies, it is an important side note under the aspects of affinity to mention a potential concern for IgG4 antibodies. For IgG4 therapeutic mAbs, a reduction in affinity can sometimes be attributed to Fab arm exchange, which is the random exchange of a half-molecule (heavy chain "HC" and attached light chain "LC") with that of another IgG4 half-molecule that effectively creates monovalent bispecific antibodies. Fab arm exchange with plasma IgG4 can reduce therapeutic IgG4 affinity and efficacy because the loss of bivalency renders the resultant mAb incapable of effectively crosslinking the target antigen or undergoing multivalent target binding. Fab arm exchange is attributed to weak CH3-CH3 interactions and to relatively labile inter-HC disulfide bonds of the hinge region, which can undergo shuffling under reducing conditions in blood or at cell surfaces.[50,51] Labrijn et al.[52] observed that natalizumab, an IgG4 mAb against the α4 subunit of α4β1 (VLA-4) and α4β7 integrins, undergoes Fab arm exchange with endogenous IgG4 in humans, whereas gemtuzumab, an IgG4 mAb against CD33, which contains a core-hinge mutation, does not. The Ser228Pro mutation (IgG4S228P) stabilizes the disulfides in the core-hinge of IgG4 molecules and blocks Fab arm exchange. Stubenrauch et al.[53] investigated whether the IgG4S228P mutation would be sufficient to stabilize the core hinge region of an IgG4 mAb and prevent Fab arm exchange. Although the mutation did not completely prevent Fab arm exchange *in vitro* under reducing conditions, the process was markedly attenuated *in vivo* in cynomolgus monkeys where serum was virtually free of the Fab-swapped IgG4.

Glycosylation. Most therapeutic mAbs are N-glycosylated at the residue Asn297 in the heavy chain of the CH2 domain with the (Asn)-X-Ser/Thr (where X is any residue except a Pro) consensus sequence. Additional glycosylation can also exist in the variable region, such as the antiepidermal growth factor receptor mAb, cetuximab.[51,54,55] The N-linked glycans are composed of a conserved biantennary complex structure of N-acetylglucosamine residues (GlcNAc) and mannose (Man) residues in the core and extending terminal-sugar residues (e.g., Man, GlcNAc, galactose (Gal), core fucose, and sialic acid) added depending on the host glycosylation machinery.[54–56] The impact of glycosylation is not limited to the PK of therapeutic mAbs but extends to effector functions and PD, which will be discussed in the next section.

The prevailing assumption is that the FcRn-binding site located at the CH2–CH3 domain and variations in Fc glycosylation, specifically IgG1, may also not likely impact FcRn-mediated clearance pathways of therapeutic antibodies. The position of Fc glycans on the interior surface of the Fc domain makes them inaccessible to glycan receptors that may participate in FcRn-mediated mAb clearance, such as asialoglycoprotein and Man receptors.[55] Several studies documented a change from an "open" to a "closed" IgG conformation upon removal of glycans and have shown that it impacts binding of FcγRs but not FcRn.[57,58] Thus, no measurable or meaningful changes in PK properties are observed with most of the Fc-glycan variants (except high mannose variants) of mAbs. Leabman et al.[59] also showed that aglycosylated and afucosylated mAbs are eliminated with a similar rate compared with to glycosylated or fucose-containing mAbs, respectively.

Table 3 Selected studies on the impact of high-mannose glycoforms on mAb PK

Impact on PK	mAb	Mannose composition	References
Increased clearance	Dansyl-specific (chimeric IgG1)	Produced by Lec1 CHO cells: 100% M5	68
	CD20-specific (chimeric IgG1)	Produced by Lec1 CHO cells: 100% M5	69
		Engineered with kifunensine: 100% M8–M9	
	WT-mAb (IgG1)	Produced by *Pichia pastoris*: fungal-type high mannose glycosylation	62
	mAb1-mAb4 (human IgG1 or 2)	5–17% M5 (excluding M6–M9 to M5 *in vivo* conversion)	73
No effect on clearance	Omalizumab (humanized IgG1)	5–7% M5	70
	mAb1 (human IgG1)	Enriched for high mannose: 52% M5–M8	71
	IL1R-specific (human IgG2)	M6–M9 (% composition not specified)	72

CHO, Chinese hamster ovary; IgG, immunoglobulin G; IL-1R, interleukin 1 receptor; mAb, monoclonal antibody; PK, pharmacokinetics; WT; wild type.

Although recombinant mAbs produced in murine hybridoma cells can be up to 50% sialylated in sharp contrast to the typical 10% sialylation for human IgG, the degree of sialylation does not seem to impact PK based on a limited number of studies.[56,60] Glycan variants, such as desialylated and agalactosylated mAbs, respectively, produced in cell lines deficient in attachment of sialic acid (Lec 2) and Gal (Lec 8) were comparable to wild-type mAbs with respect to *in vivo* half-life.[61] So far, the only glycan that negatively impacts PK is high terminal Man content by increasing the clearance and the reducing half-life (**Table 3**). Wright *et al.*[62] showed that M5 IgG (produced by the glycosylation-mutant cell line Lec1, and consisting of 100% M5 glycan variants) demonstrated faster clearance in mice and tended to distribute to the liver. Similarly, Yu *et al.*[63] have shown that high-Man mAbs (such as > 99% M5, or > 99% M8/9) displayed threefold faster clearance and about twofold shorter half-lives than the IgG containing the complex glycans in mice. They also showed that higher Man variants, such M7/8/9, are converted to M5 by mannosidase.

In humans, Chen *et al.*[64] showed that the serum levels of several high-Man mAbs (M6-M9) decreased faster than other glycoforms due to their conversion to M5 by mannosidases rather than their clearance and concluded that antibody clearance in humans is not significantly affected by the Fc glycan structure. On the other hand, in a follow-up study[65] the authors from the same group used a highly sensitive analytical method (peptide mapping using high-resolution mass spectrometry) to follow changes to the glycan PK profile of four therapeutic mAbs over time and found that M5 cleared faster than other non-Man glycan variants. A twofold difference in the half-life between the variants was also reported. There are few studies in mice and rats that have reported no impact on PK of the high-Man variants.[66–68] Harris[66] showed that omalizumab (5–7% M5) has a similar PK profile to the other glycoforms in mice. Millward *et al.*[68] showed that a preparation of a mAb enriched for high mannose glycoforms (52% M5–M8) showed similar clearance rates in mice to those of the parent mAb (3% M5–M7). One of the reasons for the discrepancy between different PK studies is the reduced amount of high-Man variants present compared with the previous studies.[61,63] Overall, the published data suggest that high-Man glycoforms are cleared more rapidly than other glycoforms, and emphasize the importance of controlling the levels of Man variants, and having it as a critical quality attribute for PKs.

STRUCTURAL VARIANTS TO IMPROVE TISSUE-SPECIFIC DELIVERY

As previously noted, the focus of this review is on mAbs; however, an important exception is noted with tissue uptake with bispecifics. Recently, antibodies have been designed to improve delivery to the site of action. For example, bispecific antibodies that bound transferrin receptor and beta-secretase, with no binding to FcγRs or complement proteins, were used to improve delivery of the antibody to the brain. The binding affinity to transferrin receptor was optimized to obtain favorable brain PK/PD profiles.[45] A more recent advancement in the tissue-specific delivery of antibodies has been development of Probody Therapeutics that are designed to be activated only in the disease tissue microenvironment by taking advantage of the upregulation of protease activity in the disease tissues.[69]

IMPROVING PHARMACODYNAMICS THROUGH ANTIBODY DESIGN

The primary goals of all previously discussed factors that influence PK design was to enhance the overall activity and clinical efficacy of therapeutic mAbs. As noted, this could be through enhanced affinity and/or a tailored half-life. Engineering mAbs to improve their PDs and ultimately their therapeutic potential also involves modifying the variable region for enhancing antigen-binding activity and modifying the Fc-mediated effector function to further increase clinical potential.

Modifying effector functions

The Fc portion of mAbs contains binding sites for FcRs and complement proteins, through which mAbs (upon forming immune complexes) can initiate immune effector functions that enhance the killing and removal of mAb-bound target cells. These functions bridge the adaptive humoral immune response to innate cellular defense mechanisms, which include antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and antibody-dependent cell-mediated phagocytosis

(ADCP).[51,70] Another Fc-mediated function is the clustering effect crucial to the activity of agonistic mAbs (such as anti-CD40), in which the Fc-FcR interaction leads to higher order clustering of the antibody and, thus, of the target receptor bound by the variable domain, triggering downstream signaling.[71] However, not much progress has been made in the translation of agonistic mAbs into effective therapies for patients.

FcRs are divided into type I FcRs (generally known as FcγRs) and type II FcRs (which include CD23 and CD209) and are classified based on their binding to IgG. Type I FcRs bind the Fc in the open conformation near the hinge proximal region and with a 1:1 stoichiometry; type II FcRs bind IgG in the closed conformation in the CH2-CH3 domain with stoichiometry of two receptors per antibody. Although the engagement of type II FcRs leads to suppression of antibody-mediated and T cell-mediated inflammation, the engagement of type I FcRs results in ADCC and ADCP.[72] Recently, additional FcRs have been identified, including Fc receptor-like molecules and TRIM21. Their role is not well understood. On the other hand, binding of IgG to the type I FcRs (FcγRs) has been extensively leveraged for designing mAbs with modified effector functions and is discussed briefly here. FcγRs are further subdivided into activating and inhibitory receptors depending on their intracellular motif (**Table 4**). B cells, innate immune cells, including myeloid cells (dendritic cells, monocytes, macrophages, neutrophils, basophils, and eosinophils), and natural killer (NK) cells differentially express these receptors.[72,73] T cells do not express FcγRs,[74] although there is a recent report of FcγRIIb expression on memory CD8 T cells.[75] The outcome of the IgG-FcγR interaction is governed by several factors, such as the Fc isotype and structure, the cellular and preferential expression patterns of FcγRs, allelic variation of FcγRs, ratio of the activating vs. inhibitory receptor engagement, and species differences. Many of these factors have been studied in mice but not in humans. It is also suggested that macrophages largely drive ADCP activity via FcγRII and FcγRIII, whereas ADCC is driven by NK cells via FcγRIII only. The binding affinities of the various Fc isotypes to IgG are governed by the N-linked glycosylation pattern at Asn297 as well as the amino acid structure, both of which determine which of the two dominant conformational states the Fc domain may adopt. Consequently, glycoengineering (modifying glycosylation patterns) and protein engineering (modifying the amino acid sequence using display libraries and/or structure-guided design) have been adopted to either enhance or reduce/abrogate Fc effector function depending on the desired therapeutic activity of the mAb. Because the screening for Fc activity of IgG mAbs is performed *in vitro* (such as ADCC, ADCP, and CDC assays) as well as *in vivo* (such as antitumor efficacy in murine models or toxicology studies in nonhuman primates), the species differences in expression of FcγRs and in affinities should be taken into account, because they can complicate the contribution of Fc activity, and the clinical translation of preclinical activity.[76–78]

Enhancing Fc effector functions. Enhanced Fc-mediated effector functions are highly desirable when the removal of the mAb-targeted cell is crucial to clinical efficacy, such as tumor cells or virally infected cells. Specifically in oncology, several mAbs with potent effector functions (ADCC, ADCP, and/or CDC) are clinically approved, including rituximab, obinutuzumab, trastuzumab, and cetuximab. More recently, several next-generation antitumor mAbs optimized for Fc-mediated effector function have progressed into clinical development.[72] To induce immune cell activation, mAbs must first overcome the activation threshold dictated by the presence of both activating and inhibitory FcγRs. This is achieved by enhancing the relative engagement of the activating (most importantly FcγRIIIA) vs. inhibitory FcγRs (increasing the A-I ratio) through glycoengineering and protein engineering.[72]

Glycoengineering. The glycosylation pattern of mAbs at Asn297 regulates the conformational state of the Fc domain and subsequent binding to Fc receptors.[57] These N-glycans are crucial for the structural integrity of the FcγR-binding site, and their removal has been shown to abrogate FcγR affinity and complement binding/effector functions.[79,80] Reducing fucose and sialyl groups and increasing bisecting GlcNAc, Gal, and Man have proven to enhance Fc-mediated ADCC and/or CDC.[81]

Afucosylation has by far been the most effective glycoengineering approach to enhancing Fc effector function of mAbs. Shinkawa *et al*[82] showed that the absence of fucose, rather than the presence of bisecting GlcNAc or Gal, is necessary for maximal enhancement of ADCC activity. Furthermore, afucosylation does not affect binding to FcγRI, C1q, or FcRn.[82,83] Importantly, afucosylation enhances ADCC activity regardless of FcγRIIIA polymorphism.[84,85]

Table 4 Human IgG subclasses and binding affinity to human FcγRs[a b]

Fc receptors		IgG1	IgG2	IgG3	IgG4
Activating	FcγRI	High	No binding	High	High
	FcγRIIa$_{H131}$	Medium-low	Low	Low	Low
	FcγRIIa$_{R131}$	Medium-low	Very low	Low	Low
	FcγRIIIa$_{V158}$	Medium-low	Very low	High	Low
	FcγRIIIa$_{F158}$	Low	No binding	High	Low
Inhibitory	FcγRIIb	Low	No binding	Low	Low

IgG, immunoglobulin.
[a]The murine FcγRs that functionally correspond to the human FcγRI (hCD64), FcγRIIa (hCD32a), FcγRIII (hCD16), and FcγRIIb are FcγRI, FcγRIII, FcγRIV, and FcγRIIB, respectively. [b]C1q binding: IgG3 > IgG1 > IgG2. IgG4 does not bind C1q.

Several glycoengineered afucosylated mAbs have demonstrated clinic success, the first of which is mogamulizumab, a humanized mAb targeting C-C chemokine receptor type 4 approved for patients with relapse or refractory C-C chemokine receptor type 4-positive T cell leukemia or lymphoma.[86] Another example is obinutuzumab, an afucosylated anti-CD20 mAb that contains a bisecting GlcNAc. Obinutuzumab was approved in 2013 for the treatment of patients with previously untreated chronic lymphocytic leukemia. Compared with rituximab, obinutuzumab displays 50-fold higher affinity to human FcγRIII, 10–100-fold increase in ADCC against CD20-expressing lymphoma cell lines, and superiority in clinical trials for patients with chronic lymphocytic leukemia.[87,88] A third example is benralizumab, a humanized afucosylated anti-IL-5Rα that mediates ADCC depletion of IL-5Rα-expressing eosinophils implicated in asthma exacerbations. Reed *et al.*[89] showed that a single intravenous dose of benralizumab (MEDI-563) led to a robust depletion of eosinophils in mild asthmatics that was sustained for 8–12 weeks. High mannose content (Man5/8/9) has been associated with affinity for FcγRIIIA and enhanced ADCC activity, but this may rather be due to afucosylation, because high-mannose glycans do not have core fucosylation similar to bisecting GlcNAc-variants.[63,90]

Protein engineering. Another approach to enhance Fc effector functions is through introduction of Fc domain point mutations. This approach is not limited to FcγRIII and allows manipulating the interactions of the Fc domain with multiple activating FcγRs. A series of Fc variants of therapeutic mAbs (rituximab, cetuximab, trastuzumab, and the anti-CD52, alemtuzumab) were engineered and showed increased FcγRIIIA affinity, reduced FcγRIIB affinity, and enhanced ADCC activity for the double mutant Ser239Asp/Ile332Glu and the triple mutant Ser239Asp/Ile332Glu/Ala330Leu.[91] Several preclinical studies demonstrated the enhanced *in vivo* activity of Fc protein-engineered mAbs against cancer and viruses,[92,93] and a number of these mAbs have entered clinical development, such as anti-CD19 Xmab5574 for leukemia, anti-CD30 Xmab2513 for lymphoma, and anti-HER2 margetuximab for breast cancer.[72]

Fc protein engineering can also enhance ADCP activity using the Gly236Ala mutation, which selectively enhances affinity for FcγRIIA. The enhancement was further improved through combining the Gly236Ala mutation (the macrophage-mediated ADCP and NK cell-mediated ADCC) as well as the ADCC-enhancing Ser239Asp/Ile332Glu double mutation.[94] Mutations located in the upper hinge region can selectively enhance CDC activity by enhancing binding to C1q, such as the tryptophan substitutions.[95] Other point mutations, such as Lys326Trp and Glu333Ala, in the CH2 domain have been shown to enhance CDC.[96] Double mutants, such as Lys326Trp/Glu333Ala, Lys326Ala/Glu333Ala, and Lys326Met/Glu333Ser, as well as triple mutants, such as Ser267Glu/His268Phe/Ser324Thr, have displayed further

increases in CDC as compared with those observed with individual mutations.[96,97]

Reducing/silencing Fc effector functions. Although enhancing Fc effector functions can enhance therapeutic efficacy and/or reduce the dose or dosing frequency, it may also lead to Fc-mediated activation of immune cells and more frequent or severe adverse events in patients. Moreover, Fc effector functions can be undesirable for the required efficacy of some therapeutic mAbs, such as benign blocking, where the interaction with the antigen is sufficient and ADCC/ADCP-mediated depletion is unwarranted as with effector T cells.[98,99] To minimize or eliminate Fc effector functions, glycoengineering and protein engineering approaches have been used.

Glycoengineering. Common approaches to reduce Fc-mediated cell killing include using fucosylated mAbs and/or low-bisecting GlcNAc content. Another approach is to increase the affinity of the Fc domain to inhibitory type II FcRs using sialylation, which induces a closed conformation of Fc.[72] This property is also exploited with intravenous IgG (IVIG), which is used for the treatment of autoimmune and inflammatory diseases. IVIG preparations contain 10% Fc-sialylated IgG, to which the anti-inflammatory properties of IVIG are attributed.[100] Binding of IVIG to DC-SIGN induces the expression of inhibitory FcγRIIB on inflammatory cells, which attenuates inflammation.[101] To recapitulate the anti-inflammatory effect of IVIG, the glycoengineered addition of 2,6 sialylation to IgG1 or increasing the sialylation levels demonstrates lower affinity for FcγRIIIA and reduced ADCC potency.[102,103]

Protein engineering. In addition to glycoengineering, efforts to reduce Fc effector functions include introducing point mutations and/or using the IgG2 and IgG4 isotypes that have poor effector functions. The effect of reducing Fc effector functions and its impact on the PD profile has been explored with anti-IgE antibodies (HAE1 and HAE2). Using a single-point mutation (Asp265Ala) in the Fc region, which reduces HAE2-IgE complex binding to FcγRI, II, and III by ~85-fold as compared with HAE1, the authors observed reduced IgE-HAE2 complex clearance and increased total IgE serum concentrations in cynomolgus monkeys, suggesting the importance of effector functions in elimination of anti-IgE–IgE complexes.[44]

Besides silencing IgG1 activity, switching to a different subclass (e.g., IgG4) is also commonly used for therapeutic mAbs whose Fc effector functions are undesirable, such as natalizumab and the anti-PD-L1 mAb, BMS-936559. Both mAbs are designed to inhibit leukocyte interactions implicated in disease pathology without eliminating the leukocytes through Fc-mediated depletion. It is important to note that effector functions are not completely attenuated with the IgG4 subclass. Another isotype-based approach is the use of hybrid IgG2-IgG4 Fc domains in mAbs. Examples include eculizumab, which targets the complement component C5. To avoid proinflammatory responses induced by complement activation or Fc-expressing inflammatory cells, components of both human IgG2 and IgG4 constant regions

were included, because IgG2 does not bind Fc receptors as well as IgG1, and IgG4 does not activate the complement cascade.[104] Other examples include r18D11, which blocks CD14 and is used to attenuate inflammatory responses. The human IgG2/IgG4 hybrid C region was used to remove undesired Fc-mediated functions, such as platelet activation and IL-8 release.[105] Combining point mutations with IgG2/ IgG4 subclasses has also been examined. One example is IgG2 with four-point mutations (His268Gln/Val309Leu/Ala330Ser/ Pro331Ser) derived from IgG4.[106] It is worth noting that most of these approaches do not completely abrogate Fc effector function. For example, Arce Vargas et al.[77] showed that both human IgG1 and IgG2 anti-CTLA-4 mAbs showed similar regulatory T-cell depletion in mice. Complete silencing has been demonstrated by some groups.[107,108] Vafa et al.[108] utilized a combination of an IgG2 backbone and seven-point mutations (Val234Ala/Gly237Ala/Pro238Ser/His268Ala/ Val309Leu/Ala330Ser/Pro331Ser) to induce structural perturbations of the Fc and completely "mute" it by eliminating affinity for FcγR and C1q. Importantly, the affinity to FcRn was not affected, and a circulating half-life similar to that of IgG1 and IgG2 was observed in cynomolgus monkeys.

Modifying activity. The biological activity of mAbs is a mainstay of the PD profile and is chiefly determined by the variable region through which the mAb interacts with its target. In addition to improving the PD profile of mAbs through engineering the Fc-mediated effector functions, enhancing the biological activity of mAbs can be achieved by improving the design of the variable region through affinity maturation and through modulating glycosylation.

Affinity maturation. Enhanced affinity can translate into better potency, enhanced biological activity, and lower doses. For example, Ahmed et al.[109] matured the affinity of an anti-B7-H3 mAb (8H9) that is used for radioimmunotherapy of B7-H3-positive tumors using sequential random mutagenesis. The authors achieved twofold to ninefold enhancement in affinity, which translated into higher tumor uptake in mouse xenografts and improved ADCC activity. However, this may not always be true, as observed in several studies. For example, Rudnick et al.[110] studied six different anti-HER2 IgG variants that differ in single-point mutations in the CDR region affecting their affinity. The authors observed that higher affinity did not always translate into enhanced tumor targeting but rather promoted mAb internalization and degradation, leading to limited tumor penetration. Interestingly, the different affinity variants showed differences in spatial distribution within the tumor. Their studies also emphasized that tumor antigen expression levels, antigen recycling rates, antibody-antigen dissociation rate, together with the affinity impacted antibody uptake and distribution, and, therefore, activity. In another interesting study by the same group, Tang et al.[111] showed that the affinity to the tumor antigen HER2 impacted the extent and efficiency of ADCC. Additional data are necessary to fully understand the role of affinity in tumor uptake, distribution, and activity. Furthermore, these studies highlight the challenge in translating in vitro studies of biological affinity into in vivo targeting and ultimately, clinical efficacy.

CONCLUSIONS

Optimization of the PK/PD properties of therapeutic mAbs is an ongoing effort not only for novel mAbs still in the pipeline but also for approved mAbs. In this review, we have discussed the PK and PD properties of antibodies, pinpointed challenges, and highlighted approaches undertaken to overcome these challenges. Notwithstanding the interdependence of these properties, other issues instrumental to improving mAb design include the need for better understanding of the structural variants of mAbs and how heterogeneity ultimately affects mAb design and function. In addition, better understanding of the role of FcRn in IgG bioavailability, tissue uptake, and of the impact of non-FcRn binding regions on FcRn binding can provide potential for enhancing PK properties through FcRn-dependent antibody engineering. Another important issue is the mechanistic understanding of the impact of mAb affinity to the different FcγRs, and the role of FcγR-expressing immune cells in mediating the therapeutic efficacy of mAbs, particularly those being designed for cancer therapy. Lastly, novel mAb formats, such as sweeping antibodies, YTE mutants, afucosylated mAbs, and bispecifics, such as T-cell engagers, have expanded the repertoire of design tools and supplied new opportunities for designing mAbs with optimal clearance, extended serum half-life, enhanced Fc functions, and pharmacological activity. New efforts in developing a quantitative structure-activity relationship model[112,113] for predicting human clearance for antibodies as routinely done with small molecule drugs may overcome the slow progress in mAb optimization and revolutionize the field of PK/PD design of antibody therapeutics. Nonetheless, additional robust technologies and tools (both experimental and in silico) are still critically needed to understand structural determinants of the mAb that impact its PK/PD properties and use these determinants to predict the PK/PD properties and guide antibody optimization.

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